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# Commonly circulating human coronaviruses do not have a significant role in the etiology of gastrointestinal infections in hospitalized children

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ABSTRACT

*Background:* Human coronaviruses (HCoVs) OC43, 229E, NL63 and HKU1 are common causes of respiratory infections. Over the years, it has been proposed that HCoVs play a possible role in gastrointestinal infections.
*Objectives:* To assess the role of HCoVs in acute gastroenteritis (AGE) in children.
*Study design:* Study was conducted at Tampere University Hospital over 2 years. Both stool and nasal swab samples were collected from 172 children with AGE, 545 with acute respiratory tract infection (ARTI) and 238 with symptoms of both. The samples were tested for HCoVs by RT-PCR.
*Results:* HCoVs were detected in 52 (5.4%) children: in 6.4% of those with AGE, 4.4% with ARTI and 7.1% with symptoms of both. HCoVs OC43, HKU1, 229E and NL63 were encountered in 13, 11, 13 and 15 cases, respectively. HCoVs were detected simultaneously in stool and nasal swab samples in 17 children, in nasal swabs alone in 33 children, and in the stools alone in two children. HCoVs were present in the stools of eight (4.7%) of the 172 children with AGE; in six of these cases, the nasal swab sample was also positive for the respective HCoV. Additionally, in six of the eight cases, the stool sample contained either rotavirus or calicivirus.
*Conclusions:* HCoVs can be detected in the stools of children with AGE, but usually together with well-known gastroenteritis viruses, and concomitantly in the respiratory tract. It appears that commonly circulating HCoVs do not have a significant role in the AGE of children admitted to hospital.


## 1. Background and objectives

Human coronaviruses (HCoV) cause respiratory infections of varying severity. OC43 and 229E, the "common cold viruses", have been known since the 1960s [1–4], and they have been found in 1.9% of children hospitalized for respiratory tract infection [5]. A more recently discovered coronavirus, NL63 [6], has been detected in 1.7–3.2% of respiratory tract samples of children hospitalized with acute respiratory tract infection (ARTI) [5,7–9], including acute laryngitis [5,7,9]. Another recently discovered human coronavirus, HKU1 [10], was detected in 2.95% of children hospitalized for upper or lower respiratory tract infections [11]. It seems that all of these HCoVs may cause respiratory tract infections in children that are severe enough to lead to hospitalization, but none of them seems to be more pathogenic than any other [12].

HCoVs can also cause serious disease, as shown by SARS-CoV experience [13–16] and the recently discovered MERS-CoV, which causes severe respiratory infection and renal dysfunction [17]. Both of these coronaviruses are of animal origin and are not well established in humans.

Electron microscope (EM) studies in the 1970s detected coronavirus-like particles in the stools of children with acute gastroenteritis (AGE), and the existence of enteric coronaviruses was proposed [18]. The possibility that HCoVs could have a role in gastrointestinal infections is supported by findings that some animal coronaviruses are "pneumoenteric" and capable of causing both gastrointestinal and respiratory tract infections [19]. When SARS-CoV was discovered in 2003, it was noted that 23.6–73% of SARS patients had diarrhea [16,20,21], SARS-CoV RNA could be detected in the patient's stools [14,16,21], and SARS-CoV could be isolated by culture in the intestinal tissues [21].

Gastrointestinal symptoms may also occur in patients with non-SARS-CoVs, but usually HCoVs have been studied only from respiratory samples [22] or, if found in stool samples, the

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**Table 1**
Characteristics of the study groups.

| | N | Male (%) | Age (median) | Number of children in the different age groups | | | | Number of cases per season | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | <6 months | 6–24 months | 2–5 years | >5 years | 2009–2010 | 2010–2011 |
| All patients studied | 955 | 62.3 | 14 months | 224 (23.5%) | 481 (50.4%) | 178 (18.6%) | 72 (7.5%) | 559 (58.5%) | 396 (41.5%) |
| AGE[a] group | 172 | 61.6 | 20 months | 31 (18.0%) | 63 (36.6%) | 48 (27.9%) | 30 (17.4%) | 92 (53.5%) | 80 (46.5%) |
| ARTI[b] group | 545 | 62.9 | 13 months | 156 (28.6%) | 275 (50.5%) | 93 (17.1%) | 21 (3.9%) | 352 (64.6%) | 193 (35.4%) |
| AGE/ARTI[c] group | 238 | 61.3 | 13 months | 37 (15.5%) | 143 (60.1%) | 37 (15.5%) | 21 (8.8%) | 115 (48.3%) | 123 (51.7%) |

[a] Acute gastroenteritis.
[b] Acute respiratory tract infection.
[c] Symptoms of both AGE and ARTI.

simultaneous presence of gastrointestinal and respiratory symptoms has made the interpretation of the results difficult [23,24]. In our previous study, we found all four commonly circulating HCoVs in the stool samples of children with AGE [23]. However, half of these children also had respiratory symptoms, and some of the stool samples of the control children without gastrointestinal symptoms also harbored HCoVs.

In this prospective study, we simultaneously collected stool and nasal swab samples from children with AGE and an ARTI in order to clarify whether HCoV findings in stools are actually associated with AGE.

## 2. Study design

### 2.1. Patients and samples

This study was approved by the Ethics Committee of Pirkanmaa Hospital District and conducted at Tampere University Hospital's Department of Pediatrics from September 2009 to August 2011.

Children under 16 years of age with AGE who were admitted as outpatients or inpatients, or who came down with AGE during a stay in hospital, were eligible for the study. Of children with ARTI, those admitted as inpatients were eligible. Informed consent was obtained from the parents of all children enrolled. For the analysis of the study results, the patients were divided into three groups: the AGE group (children with symptoms of gastrointestinal infection only), the ARTI group (children with symptoms of respiratory tract infection only), and the AGE/ARTI group (children with different combinations of symptoms of both AGE and ARTI). Some children were admitted to the hospital more than once during the study period, and these admissions were considered to represent separate episodes if the child had been healthy for at least 2 weeks between the admissions.

Altogether, 1610 patients were eligible for the study, but in only 955 cases were both stool and nasal swab samples available and tested for HCoVs. These 955 cases included 172 children with AGE, 545 with ARTI and 238 with symptoms of both AGE and ARTI (Table 1). Moreover, 288 acute phase serum samples were available from these 955 children.

In addition to HCoVs, all stool and nasal swab samples were examined for human bocaviruses [25], all stool samples were examined for rotaviruses [26], and the stool specimens of the patients in the AGE and AGE/ARTI groups were examined for caliciviruses (including noroviruses and sapoviruses) [26,27]. HCoV-positive stool samples were additionally tested for adenoviruses and astroviruses.

### 2.2. Methods

Stool specimens were diluted in phosphate-buffered saline to create 10% suspensions. Nasal swab specimens were collected in UTM-RT Mini tubes (Copan Italia, Brescia, Italy), blended, centrifuged, and used for extraction.

A QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) was employed to extract viral nucleic acid from stool suspensions, nasal swabs and sera. The nucleic acid was amplified by a two-step RT-PCR method as described previously [23]. Primers covered a part of the conserved polymerase gene region and were designed to recognize all HCoVs in the first PCR step, and more specifically group 1B (229E and NL63, now classified as alphacoronaviruses), group 2A (OC43 and HKU1, now lineage A of betacoronaviruses) and SARS-CoV in the second step. PCR products were recognized in agarose gel electrophoresis and the HCoV types were finally determined by sequencing.

For the other viruses studied, RT-PCR was used for rotaviruses and caliciviruses [26,27], PCR was used for human bocaviruses [28] and ProSpecT enzyme immunoassay kits (Oxoid, Basingstoke, UK) were used for adenoviruses and astroviruses. In addition, samples that tested positive for the adenovirus antigen were also tested using the PCR method based on Allard et al. [29] to distinguish enteric adenoviruses from non-enteric types.

IBM SPSS Statistics 20 (IBM Corp., Armonk, USA) was utilized for statistical analysis. Fisher's exact test or $\chi^2$ test were used according to the criteria for the tests, and $p$ values below 0.05 were considered statistically significant.

## 3. Results

Of the 955 children studied, 595 (62.3%) were male. The median age was 14 months, with a range from 6 days to 15 years; the age distribution is shown in Table 1.

HCoVs OC43, HKU1, 229E and NL63 were detected in 19 stool samples (2.0%) and 50 nasal swab samples (5.2%). As expected, no SARS-CoV or SARS-like CoV was detected. In all but two cases of an HCoV RNA-positive stool sample, the same coronavirus was concomitantly detected in the nasal swab sample. HCoV RNA was not detected in any of the 20 available serum samples from the HCoV-positive children.

The seasonality of the HCoV findings is shown in Fig. 1. HCoV 229E and HKU1 circulated mainly during the first season, from September 2009 to August 2010, and OC43 and NL63 during the second season, from September 2010 to August 2011.

HCoV OC43 was detected in the ARTI and AGE/ARTI groups but not in the AGE group (difference between the groups was statistically significant, $p = 0.036$; Table 2), whereas HKU1, 229E and NL63 were all found in each of the three study groups, with no significant differences between the AGE, ARTI and AGE/ARTI groups ($p = 0.486$ for HKU1, $p = 0.079$ for 229E and $p = 0.362$ for NL63; Table 2). The most commonly detected virus was HCoV NL63 (15 cases), followed by OC43 (13 cases), 229E (13 cases) and HKU1 (11 cases). HCoV NL63 was detected concomitantly in stool and nasal swab samples in seven (47%) of the positive cases, and OC43 in six (46%) of the positive cases. HKU1 and 229E were mainly detected in the nasal swab samples only.

HCoVs were detected in eight (4.7%) stool samples of children in the AGE group, and in six of the eight cases, both stool and nasal

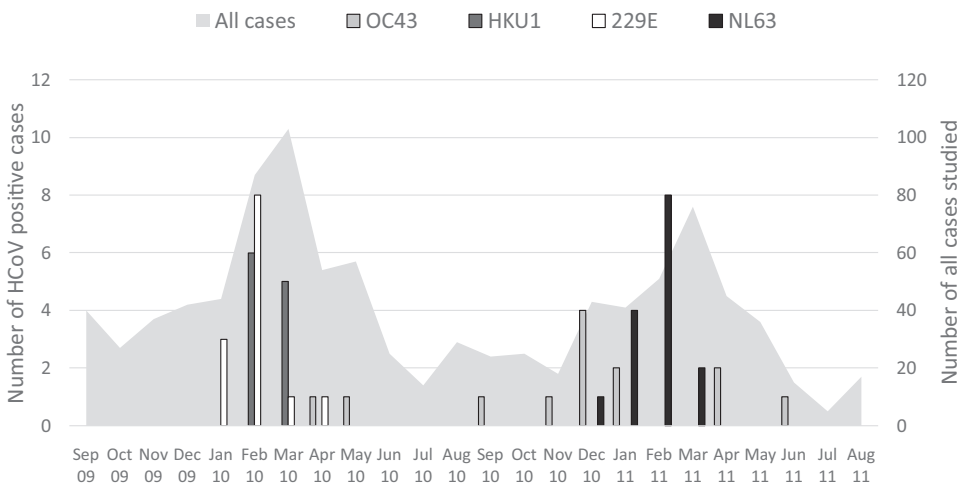


**Fig. 1.** Monthly distribution of human coronavirus (HCoV) positive cases and all cases studied.

**Table 2**
HCoV findings in the different study groups.

| | AGE[a] | ARTI[b] | AGE/ARTI[c] | *p*-value[d] |
|---|---|---|---|---|
| Stool samples | | | | |
| HCoV-OC43 | 0 | 2(0.4%) | 4(1.7%) | 0.079 |
| HCoV-HKU1 | 1(0.6%) | 1(0.2%) | 0 | 0.390 |
| HCoV-229E | 3(1.7%) | 0 | 0 | 0.006 |
| HCoV-NL63 | 4(2.3%) | 2(0.4%) | 2(0.8%) | 0.036 |
| Nasal swab samples | | | | |
| HCoV-OC43 | 0 | 6(1.1%) | 7(2.9%) | 0.036 |
| HCoV-HKU1 | 2(1.2%) | 8(1.5%) | 1(0.4%) | 0.486 |
| HCoV-229E | 4(2.3%) | 4(0.7%) | 4(1.7%) | 0.183 |
| HCoV-NL63 | 3(1.7%) | 6(1.1%) | 5(2.1%) | 0.425 |
| Combined results[e] | | | | |
| HCoV-OC43 | 0 | 6(1.1%) | 7(2.9%) | 0.036 |
| HCoV-HKU1 | 2(1.2%) | 8(1.5%) | 1(0.4%) | 0.486 |
| HCoV-229E | 5(2.9%) | 4(0.7%) | 4(1.7%) | 0.079 |
| HCoV-NL63 | 4(2.3%) | 6(1.1%) | 5(2.1%) | 0.362 |

[a] Acute gastroenteritis.
[b] Acute respiratory tract infection.
[c] Symptoms of both AGE and ARTI.
[d] Fisher's exact test.
[e] Positive stool and/or nasal swab sample.

swab samples were positive for the same HCoV. Additionally, in six of the eight cases another virus, rotavirus (three cases) or calicivirus (three cases), was also present in the same stool sample. In one child, an HCoV (HCoV 229E) was detected in the stool sample without other viruses and without a positive nasal swab sample. This 2-year-old boy was hospitalized overnight and received intravenous rehydration.

In the ARTI group, HCoV RNA was found in 24 children (4.4%); in 19 cases, it was only found in the nasal swab and in five cases, it was concomitantly in stool sample and nasal swab.

In the AGE/ARTI group, 17 children (7.1%) were positive for HCoVs. Both the stool and nasal swab samples were positive in six children, and the nasal swab was positive alone in 11 children. In five children with a positive stool sample, a known gastroenteritis virus (calicivirus in three cases, astrovirus in one and both in one) was detected in the same sample.

## 4. Discussion

The existence of human enteric coronaviruses has been proposed since the early EM findings of coronavirus-like particles in the stools of children with acute diarrhea, even though it was difficult to culture these coronaviruses from stools [18]. With the availability of RT-PCR methods, HCoVs have been detected in stools by the authors and others [23,24]. In our previous study, we found HCoVs OC43, 229E, NL63 and HKU1 in children with AGE, but some of the control children without gastrointestinal symptoms also harbored HCoVs in their stools [23]. The present prospective study was designed to expand on the previous one and to compare the simultaneous presence of HCoVs in stools and the respiratory tract.

All four commonly circulating HCoVs were detected in this study. With the exception of HCoV OC43, which was not detected in children with AGE only, all HCoVs were detected without significant differences in the three study groups, and all were more commonly detected in nasal swab samples than in stool samples. Therefore, none of the four coronaviruses could be specifically associated with AGE. Moreover, when HCoV was found in stools, it was almost always found concomitantly in the nasal swab too. These findings could mean that the detection in stools reflects the presence of HCoV in the respiratory tract and the virus detected in stools may only be there as the result of being swallowed. In the present study, in almost all cases of HCoV detection in both stool and nasal swab samples, the nasal swab samples were positive in the first PCR step. This suggests a relatively large quantity of viruses in the respiratory tract, compared to cases with a positive nasal swab sample only; in these cases, over half of the samples were positive only in the second PCR step. This could mean that higher virus load in the respiratory tract results in the presence of the virus in the stool either due to swallowing or by other mechanism. In most of the HCoV-positive stool samples, only the second PCR step was positive, indicating a relatively small quantity of viruses.

However, it has been shown that SARS-CoV can be isolated by culturing intestinal tissue samples and the intestinal biopsy specimens showed the presence of active viral replication in the intestines of patients with SARS [21], so the presence of SARS-CoV RNA in the stool is not only a consequence of the passive shedding of the virus from the respiratory tract. This is of interest at least for HCoV NL63, which uses the same ACE-2 receptor as SARS-CoV for cellular entry [30].

None of the sera studied was positive for HCoV RNA. These findings suggest that viremia is not common in HCoV infections, supporting the results of a Slovenian study in which whole blood samples of all HCoV-positive children (on the basis of a stool or nasopharyngeal sample) were negative [24].

To conclude, the four commonly circulating human coronaviruses, representing alphacoronaviruses and lineage A of the betacoronaviruses, can occasionally be found in stool samples of

children with AGE, but in almost all cases the virus is simultaneously detected in the respiratory tract, which is likely the source of the virus. HCoV findings in stools are more likely to be derived from respiratory infection, and the gastroenteritis symptoms can be explained by the well-known gastroenteritis viruses found in the same stool samples. Therefore, it seems that commonly circulating HCoVs do not have a significant role in the etiology of gastrointestinal infections in hospitalized children.

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## Competing interest

None declared.

## Ethical approval

The study was approved by the Ethics Committee of Pirkanmaa Hospital District (ETL code: R09070).

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